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Evaluation of Antimicrobial activity of Volatile Oil and total Curcuminoids extracted from Turmeric

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Abstract: Volatile oil content in dried powder of *C. longa* rhizome was investigated by hydrodistillation and total curcuminoids content was evaluated by a UV spectrophotometer . Average yields of volatile oil and total curcuminoids in dried turmeric powder were 8.20 ±1.66 %v/w and 7.57 ± 0.04 %w/w, respectively.
Volatile oil and total curcuminoids were evaluated for antibacterial activity by Agar diffusion method against medically important bacteria viz. *B. subtilis, K. pneumonia, E. coli, Enterobacter aerogenes, Pseudomonas aeruginosa, S. auresus* and *P. mirabilis* .
Two fungi were selected viz. *Aspergillus niger* and *Candida albicans* for antifungal potential of volatile oil and total curcuminoids extracted from *C.longa* L. by Agar well diffusion method.
Total curcuminoids content showed better antibacterial as well as antifungal activity as compare to volatile oil extracted from turmeric. Kanamycin was used as standard drug for antibacterial activity and Fluconazole as standard for antifungal activity.
Key words: *Curcuma longa*, volatile oil, total curcuminoids, hydrodistillation, UV spectrophotometry, antibacterial activity, antifungal activity.

INTRODUCTION

Now a days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases[1,2]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions[3]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance[4], there is a constant need for new and effective therapeutic agents[5]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants[6,7]. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the worlds[8,9].

Curcuma longa L.(turmeric) is a medicinal plant that botanically is related to Zingiberaceae family[10]. Turmeric powder, derived from the rhizome of *Curcuma longa*, is commonly used as a spice, food preservative, and food-coloring agent[11,12,13] . It also has a long history of therapeutic uses. Turmeric extract is an oleoresin consisting of a volatile oil (light) fraction and a yellow–brown colour (heavy) fraction. It contains a number of curcuminoids, monoterpenoids and sesquiterpenoids. The compounds showing yellow colour are three curcuminoid compounds; curcumin,

demethoxycurcumin and bisdemethoxycurcumin. Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Diferuloylmethane], a yellow bioactive pigment, is the major component of turmeric[13,14,15] . It has been shown that curcumin have a wide spectrum of biological activities such as antifungal[10], antidiabetic[11], antioxidant[13,14], anti-inflammatory[16,17],anticancer[18],antiallergic[19],antiprotozoal [20] and antibacterial activities[10,12,21].

The volatile oil of *C. longa* reported for antiinflammatory[22], antibacterial[23,24] and antifungal[24] activities.

The present study has been focused to extract volatile oil and total curcuminoids content from turmeric(*C.longa*) for their antimicrobial properties.

MATERIALS AND METHODS:

Plant Materials: The rhizome of *C.longa* L.(turmeric) were collected from Haridwar (Uttarakhand) .The sample was identified by qualified Taxonomist .

Preparation of turmeric rhizome: Fresh rhizomes were cleaned and cut into small pieces and air dried for 2 days. The dried sample was again dried in a hot air oven at 50°C for 24 hrs., then ground into powder and pass through a sieve with nominal mesh size of 2 mm. in diameter.

Determination of total curcuminoids and volatile oil contents in turmeric powder[25-28]

Plant Materials: Dried turmeric powder was extracted for volatile oil and total curcuminoids contents.

- First of all turmeric powder (10.00gm.) was put in a 500 ml. round bottom flask. The distilled water (100 ml.) was added. The mixture was distilled at a rate of 2-3 ml/min for 05 hours. The percentage of volatile oil in the dried powder was calculated.

-Extraction with 95% ethanol after hydro-distillation of volatile oil:

After extraction of volatile oil by hydro-distillation, the marc was further macerated with 600 ml. of 95% ethanol on a shaker with 210 rpm at room temperature until the last extract was colorless . The ethanol extracts were combined and filtered. The filtrates were concentrated under reduced pressure at 50° C using a rotary vacuum evaporator. The extract was further evaporated on a boiling water bath until constant weight was obtained.

-Extraction with methanol after hydro-distillation of volatile oil:

After extraction of volatile oil by hydro-distillation, the marc was further macerated with 600 ml.of methanol on a shaker with 210 rpm at room temperature until the last extract was colorless . The methanol extracts were combined and filtered. The filtrates were concentrated under reduced pressure at 50° C using a rotary vacuum evaporator. The extract was further evaporated on a boiling water bath until constant weight was obtained.

Determination of total curcuminoids by UV spectrophotometry

***Instrumentation and analytical conditions :**

The UV method was performed using Perkin-Elmer Spectrophotometer in the visible range at 420 nm using a 1.0 cm. quartz cell. Total curcuminoids content was calculated using
a standard curve . Analysis of each sample was done in triplicate.

***Preparation of the standard solution :**

Standard curcumin(2.0 mg.) was accurately weighed and transferred to a 5 ml. volumetric flask, methanol was added and the solution was adjusted to a final concentration of 400µg/ml. From this solution, concentration of 0.8, 1.6, 2.0, 2.4 and 3.2µg/ml were prepared and used for preparation of the calibration curve.

***Preparation of the sample solution :**

The ethanol / methanol extract (10mg.) was transferred to a 10 ml volumetric flask. The sample was dissolved in methanol and adjusted to a concentration of 1.0 mg/ml. Aliquot of this solution was diluted with methanol to make the final concentration of 5.0µg/ml. The content of total curcuminoids was expressed as gram per 100 grams of the extract.

***Qualitative Anti-bacterial Studies[29-31]**

Materials and method used

Method followed: - Agar Diffusion Method

Requirements: - Petridishes, glass syringes, cork borers (all sterilized by dry heat)

Working procedure:-

Preparation of test and standard solutions:-

The test solutions of the extracts were prepared in distilled DMSO at a concentration of 1, 5 and 20 mg / ml. Kanamycin was used as standard and was dissolved in distilled DMSO to get a final concentration of 30 µg / ml. DMSO (0.1 ml) was used as solvent control.

Microorganisms used:-

The *Bacillus subtilis* (NCIM 2439), *Klebseilla pneumonia* (NCIM 2065), *Escherichia coli* (NCIM 2345), *Enterobacter aerogenes* (NCIM 2340), *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus auresus* (NCIM 2200) and *Proteus*

mirabilis (NCIM 2241) strains were employed for the present study. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

Preparation of Inoculums :-
The suspensions of all the organisms were prepared as per Mac-Farland Nephelometer Standard (Baily and Scott 1990). A 24 hrs old culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted.

Culture medium
The following media were used for the antimicrobial studies :
Nutrient broth : 37 gm of readymade powder was dissolved in 1 ltr of distilled water; pH was adjusted to 7.8 and sterilized by autoclaving at 15 lbs for 15 min.

Nutrient Agar: The sterilized medium was cooled to 40º C and poured into petridishes to obtain 4-6 mm thickness. The media was allowed to solidify at R.T (room temperature).

Sterilization:-
Sterilization of media, peptone water, distilled water etc., was carried out by autoclaving at 15 lbs for 20 min. The glassware was sterilized by dry heat in an oven at a temperature of 160 ºC for one hour .

Procedure:-
A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 ml of inoculums (of 10^4 to 10^6 CFU / ml population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 ml) solution of each sample and standard samples were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 hrs. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for extracts. The diameter of the zone of inhibition was measured and recorded .

- Qualitative Anti-Fungal Studies[32-38]
The in-vitro antifungal activity by agar well diffusion method was standardized using fluconazole. This method is based on diffusion of antifungal component from reservoir hole to the surrounding inoculated Sabouraud dextrose agar medium, so that the growth of fungus is inhibited as zone around the hole. Two fungi were selected viz. *Aspergillus niger* and *Candida albicans.*

Method followed: Agar well diffusion method
Requirements: Petri dishes, glass syringes, cork borers (all sterilized by dry heat).
Nutrient Medium: Sabouraud's Dextrose agar medium (Hi media)
Fungi used:
Standard cultures of *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 545) were employed for the present study. The fungi were maintained by sub culturing and used at regular intervals.
Sample preparation
Samples were dissolved in DMSO to get final concentrations of 5mg/ml, 10mg/ml & 15mg/ml.
Culture medium
Sabouraud dextrose agar medium (Hi Media) was used for preliminary antifungal activity. The medium was prepared by dissolving in water and autoclaving at 121^0 C for 15 minutes.
Standard Preparation
Fluconazole standard was prepared at a final concentration of 10µg/ml in sterile distilled water.
Preparation of inoculum
The suspension of fungus was prepared as per Mac-Farland Nephlometer Standard. A 24 hour –old culture was used for the preparation of fungus suspension. A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately 1.5 X 10^6 cells / ml. It was obtained by adjusting the optical density (650 nm) equal to 1.175 % barium chloride in 100 ml of 1% sulphuric acid.

Working procedure
An inoculum was prepared by suspending a single isolated colony in about 5ml of 0.9 %w/v of Normal saline. This is mixed slowly to achieve a smooth suspension. Later one drop of Tween-20 was added for filamentous fungi and the mould was broken by shaking. A sterile cotton swab was moisten in the inoculum suspension and excess of was removed by rolling the cotton swab on the inside of the tube, above fluid level 30 ml of sterile hot sabouraud's agar medium was poured in each plate and allowed to harden on a level surface. A glass spreader was moistened in the adjusted inoculums suspension and surfaces of sabouraud dextrose agar plates were streaked in 4 different directions (at 90 º angels), so as to cover the entire surfaces. Using flamed sterile borer the medium was bored and the

prepared extracts of three concentrations were taken and 0.1 ml each extract was added in each bore. This procedure was carried out for the both fungi viz. *Candida albicans* and *A. niger*. The surface of Sabouraud's agar plate was dried out 35 ^{0}C. Later bores were made using sterile cork borer. The above operation was carried out in aseptic condition and 0.1 ml test solution was added to the respective bore and 0.1 ml Fluconazole was taken as standard reference. A control having only DMSO was maintained in each plate. The plates were incubated at 35 ^{0}C for 48 hr. Later the values of zones of inhibition were recorded.

All the samples showing maximum zone of inhibition with concentration 15 mg/ml.

RESULT AND DISCUSSION

Table 1 : Weight of dried rhizome collected from Haridwar(Uttarakhand)

Location	Weight of dried rhizome(% fresh weight)
Haridwar(Uttarakhand)	20.80 ± 1.19*

* %Yield were expressed as mean ± SD (n=3)

Table 2: Extraction conditions of each extraction method after hydro-distillation

Extraction method	Time consumed (in hrs.)	Temperature (° C)	Solvent used(in ml.)		Characteristics
			95%Ethanol	Methanol	
01	310	Room Tem.	600	----	Brown powder
02	310	Room Tem.	----	600	Brown powder

01- Extraction with 95% ethanol after hydro-distillation using dried powder
02- Extraction with methanol after hydro-distillation using dried powder

Table 3 :The content of volatile oil and total curcuminoids in the rhizome of *C.longa*

Location	Volatile oil content (%v/w) in dried powder	Total curcuminoids content (%w/w) in dried powder
Haridwar(Uttarakhand)	8.20 ±1.66 *	7.57 ± 0.04 *

* %Yield were expressed as mean ± SD (n=3)

Table 4: Antibacterial activity (zone of inhibition in mm.) *

Extracted portion used	***B.subtilis***	***K.pneumonia***	***E.coli***	***Enterobacter aerogenes***	***Pseudomonas aeruginosa***	***S.auresus***	***P.mirabilis***
Volatile oil	**13**	**-----**	**11**	**08**	**-----**	**10**	**12**
Curcuminoids	**19**	**15**	**18**	**15**	**14**	**17**	**18**
Kanamycin	**23**	**21**	**22**	**22**	**20**	**24**	**21**
Control (DMSO)	**-----**	**----**	**-----**	**-----**	**-----**	**-----**	**-----**

---- = No Activity , * Average of three readings

Table 5 : Antifungal activity (zone of inhibition in mm.) *

Extracted portion used	*A. niger*	*C. albican*
Volatile oil	**12**	**14**
Curcuminoids	**17**	**18**
Fluconazole	**23**	**22**
Control (DMSO)	**-----**	**-----**

---- = No Activity, * Average of three readings

The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health .

Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine,a natural blueprint for the development of a drug .

Volatile oil content in dried powder of *C. longa* rhizome was investigated by hydrodistillation and total curcuminoids content was evaluated by a UV spectrophotometer . Average yields of volatile oil and total curcuminoids in dried turmeric powder were 8.20 ±1.66 %v/w and 7.57 ± 0.04 %w/w, respectively as shown in Table 3.

The results of antibacterial activity of Volatile oil and total curcuminoids ,extracted from *C. longa* rhizome , against the investigated bacterial strains are shown in Table 4 .

Out of Volatile oil and total curcuminoids ,extracted from *C. longa* rhizome, total curcuminoids showed more promising antibacterial activity against all bacteria tested.
The results of antifungal activity of Volatile oil and total curcuminoids ,extracted from *C. longa* rhizome , against *Aspergillus niger* and *Candida albicans* are shown in Table 5 .

Out of Volatile oil and total curcuminoids ,extracted from *C. longa* rhizome, total curcuminoids showed higher drgree of antifungal activity against both the fungal strains tested.

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